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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR
09/662,462	09/15/00	SMITH

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HM22/0410

ART UNIT	DOCKET NO.
1654	

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev.11/00)

1- File Copy

Office Action Summary	Application No. 09/662,462	Applicant(s) SMITH ET AL.	
	Examiner Jeanine A Enewold Goldberg	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 February 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23* is/are pending in the application.

4a) Of the above claim(s) *12-18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11 and 19-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) 7 .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed February 26, 2001. Currently, claims 1-23 are pending.

2. Claims 1-11, 19-23 have been examined on the merits.

Election/Restrictions

3. Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-11, 19-23, drawn to methods to detect and identify *Candida* using SEQ ID NO: 1-13 and SEQ ID NO: 33-38, classified in class 435, subclass 6.

II. Claims 1-3, 12-16, 19-23, drawn to methods to detect and identify *Aspergillus* using SEQ ID NO: 18-27 and 40-42, classified in class 435, subclass 6.

III. Claims 1-3, 17, 19-23, drawn to methods to detect and identify *Cryptococcus neoformans* using SEQ ID NO: 14-17, classified in class 435, subclass 6.

IV. Claims 1-3, 18-23, drawn to methods to detect and identify *Pneumocystis carinii* using SEQ ID NO: 28-32, classified in class 435, subclass 6.

4. It is noted that numerous claims appear in more than one group. The claims which appear in multiple groups will be examined to the extent of the species elected.

For example Claim 1 would be examined in Group IV to the extent that it applies to *Pneumocystis carinii.*

5. The inventions are distinct, each from the other because of the following reasons:

The inventions of Group I-IV are patentably distinct methods because they each have different objectives, different uses, different reagents and different method steps. The method of Group I is for methods to detect and identify *Candida* using SEQ ID NO: 1-13 and SEQ ID NO: 33-38. The methods of Group II are methods to detect and identify *Aspergillus* using SEQ ID NO: 18-27 and 40-43. The methods of Group III are methods to detect and identify *Cryptococcus neoformans* using SEQ ID NO: 14-17. Finally, the methods of Gorup IV are methods to detect and identify *Pneumocystis carinii* using SEQ ID NO: 28-32. All of these methods are directed to detecting different species using different probes. Therefore the methods are distinct over one another.

6. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their divergent subject matter, restriction for examination purposes as indicated is proper.

Response to Arguments

The response traverses the restriction. The response asserts that all of the claimed subject matter is in 435/6 indicating that the claims are not distinct. This argument has been reviewed but is not convincing because 435/6 is a class which

contains over 7,280 patents. 435/6 encompasses any nucleic acid assay which involved an enzyme. The instant groups are all directed to different genus of organisms which are not related. The search for the genuses are not coextensive and are patentably distinct. The instant groups as set out above are patentably distinct.

The response filed a petition. However, since the restriction had not been made FINAL, a petition is not timely.

The initial restriction requirement has been modified because the examiner did not deem a search of all of the Candida species burdensome.

Therefore, this restriction is made FINAL.

DETAILED ACTION

Priority

7. This application claims priority to a PCT document, a provisional and an EP document.

An application in which the benefits of an earlier application are desired must contain a specific reference to the prior application(s) in the first sentence of the specification (37 CFR 1.78).

Sequence Rules

8. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825.

Claim 3 teaches primer which are longer than 10 nucleotides and have not been designated by a SEQ ID NO:. Each of these primers need to be identified by a SEQ ID NO:.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> **The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.**

9. Claims 1-2, 19-23 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to a method of identificaiton of fungal pathogenic species using the specified probes of SEQ ID NO: 1-13, 33-38 or variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, without affecting species specific hybridization behavior of the probe".

The specification teaches the specific probes of SEQ ID NO: 1-13, 33-38 which are species specific. The specification fails to teach a probe which is specific which contains up to two mismatches 5' of the middle and/or up to two mismatches 3' of the midde.

There is not adequate description of the genus of variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one

or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, which don't affect the species specific hybridization behavior of the probe. The specification only discloses the sequences which are identified by SEQ ID NO: within the scope of the genus: variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, which don't affect the species specific hybridization behavior of the probe. The general knowledge in the art concerning variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, which don't affect the species specific hybridization behavior of the probe does not provide any indication of how to readily identify these variants. There is substantial variability among the species of nucleic acids encompassed in the scope of the claim. For example, the probes are approximately 20 nucleotides in length. If four of these bases are altered, the variant probe is only 80% identical to the original probe. The specification has also not defined a structural feature of the variants which would be common to all members of the genus that constitutes a substantial portion of the genus. Furthermore, one of skill in the art would conclude that applicant was not in possession of the claimed "variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, which don't affect the species specific hybridization behavior of the probe" because the description of only the probes identified by SEQ ID NO: of this genus is not representative of the variants of the genus

and is insufficient to support the claims. Thus, the specification does not adequately provide a written description for variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, which don't affect the species specific hybridization behavior of the probe.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 1-11, 19-23 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claims 1-10, 19, 23 are indefinite because the claims do not recite a positive process step which clearly relates back to the preamble. The preamble states that the method is for detecting and identifying a fungal pathogenic species but the final process step is merely detecting the hybridization complex. Therefore the claims are unclear as to whether the method is a method of detecting fungal pathogens or detecting hybridization complexes.

B) Claims 1-2, 19-23 are indefinite over the recitation "or variants of said probes, said variants differing from the sequences cited above by the deletion and/or addition of one or two nucleotides at the 5' and/or 3' extremity of the nucleotide sequence, without affecting species specific hybridization behavior of the probe" because it is unclear

whether the claim is intended to encompass any two base pair changes 5' of the middle of the probe and two bases 3' of the middle of the probe or whether the claim is intended to only mean adding one or two nucleotides to the 5' and/or 3' nucleotide of the probes.

C) Claims 20-22 are indefinite over the recitation "in one single assay" because it is unclear what constitutes one assay. It is unclear whether the assay is a single reaction vessels or whether a single assay is merely directed to a single experiment.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claims 1-10 (21-22) are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol 49, No. 1, pg-M23-M28) and Botelho et al. (Yeast, Vol 10, pg 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997).

Williams et al (herein referred to as Williams) teaches an alignment of *Candida albicans*, *Candida tropicalis* and *Candida krusei*. Williams teaches obtaining sequences and retrieving sequences from GenBank and EMBL for alignment using CLUSTAL V suite of programs. SEQ ID NO: 1, 2, 3, 6, 9, 33, 36 are embedded within the ITS1 region. Williams also teaches variants of SEQ ID NO: 34 –35 and 37 such that the variants differ from the sequences cited by the deletion of one or two nucleotides at the

5' extremity of the nucleotide sequence. The sequences are found in regions of variability. Williams teaches ITS1 and ITS4 and ITS1 and ITS2 as primers used to amplify DNA extracted from Candida isolates and archival tissue (limitations of Claim 3). Moreover Willaims teaches the Genbank Accession Numbers for six of the Candida species (L47111, L47112, L47114, L47109, L47113, L47107 and L47108). These Genbank Accession Numbers contain SEQ ID NO: 1-10, 33-37. Willaims specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients with CHC because of the association between this form of candidiasis and the development of oral cancer" (pg M27).

Botelho et al (herein referred to as Botelho) teaches specific identification of *Candida albicans* by hybridization with oligonucleotides derived from ribosomal DNA internal spacers. Botelho teaches an alignments of *Candida albicans* and *Candida tropicalis*. Botelho teaches that the ITS1 and ITS2 regions were found to contain distinct regions with sufficient sequence divergence to make them suitable as specific target sites for the identification of *C. albicans*. Botelho teaches that comparison of the ITS sequences was performed by computer-aided sequence comparison using the software SEQNCE and FASTA to find optimal sequence alignment. SEQ ID NO: 1, 2, 3, 6, 36 are embedded within the ITS1 region. Botelho also teaches variants of SEQ ID NO: 34 and 35 such that the variants differ from the sequences cited by the deletion of of one or two nucleotides at the 5' extremity of the nucleotide sequence. The sequences are found in regions of variability. Botelho teaches detecting and identifying

fungal pathogenic species in a sample by releasing the nucleic acids of the pathogens, amplifying the ITS with a fungal universal primer pair, hybridizing the nucleic acids with a species specific oligonucleotide probe, and detecting the complexes formed (pg 714-715). Botelho teaches that the probes which were identified unequivocally distinguish between *C. albicans* and other yeast generas as well as between .*C albicans* and other medically important Candida strains such that the have great potential as diagnostic tools (pg 715, col. 2). Botelho teaches that the ITS1 and ITS2 regions have low interspecies homology which makes them ideal probes to differentiate species.

Neither Botelho nor Williams specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of seqeunces by using a Sun Microsystme ™ computer for comparative analysis. The "compiler is capable of manipulating many sequences of data at the same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogans also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.

> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to Candida, as taught by Botehlo and Williams, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to differentiate these six Candida species for the benefit of differentiating the species from one another. Williams specifically teaches that "the ability to identify Candida within human tissue provides an opportunity to increase our knowledge of the role of candidal species in disease. This is particularly important for patients with CHC because of the association between this form of candidiasis and the development of oral cancer" (pg M27). Additionally, Botelho teaches that the probes which were identified unequivocally distinguish between *C. albicans* and other yeast generas as well as between .*C albicans* and other medically important Candida strains such that the have great

potential as diagnostic tools. Thus, the ordinary artisan would have been specifically motivated to identify and differentiate the Candida species. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. There is a reasonable expectation of success for aligning known sequences, namely *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida krusei*, and Candida glabrata, as taught by Williams using known computer alignment methods and identifying regions of variability between the six species to generate probes which are species specific, as taught by Hogan. Within the alignment provided between a select group of the Candida species provided by Williams and Botelho, the instant probes are within regions of variability. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 1-10, 33-37 are obvious.

12. Claims 1-4, 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lott et al (US Pat. 5,426,027, June 1995) and Lott (EMBL U96719, August 1997) in view of Hogan (US Pat. 5,595,874, January 1997).

Lott et al teaches detecting Candida DNA cells in blood by identifying regions of the ITS2. Lott teaches the universal primers ITS1, ITS2, ITS3, ITS4. Lott teaches sequence alightment in all possible pairwiase combinations for C. albicans, C. parapsilosi and C. tropicalis over the ITS2 region.

Lott teaches the ITS2 region of Candida dubliniensis.

Neither Lott et al or Lott specifically teaches the exact probes and primers of the instant application.

However, Hogan et al. (herein referred to as Hogan) teaches variable regions can be identified by comparative analysis of sequences by using a Sun Microsystme ™ computer for comparative analysis. The "compiler is capable of manipulating many sequences of data at the same time. Computers of this type and computer programs which may be used or adapted for the purposes are commercially available" (col. 5, lines 50-60). Hogans also teaches the use of specific primers col. 6-7, lines 50-67, lines 1-12, and furthermore provides specific guidance for the selection of primers and probes.

> "Once the variable regions are identified, the sequences are aligned to reveal areas of maximum homology or 'match'. At this point, the sequences are examined to identify potential probe regions. Two important objectives in designing a probe are to maximize homology to the target sequence(s) (greater than 90% homology is recommended) and to minimize homology to non-target sequence(s) (less than 90% homology to non-targets is recommended). We have identified the following useful guidelines for designing probes with the desired characteristics.
> First, probes should be positioned so as to minimize the stability of the probe:nontarget nucleic acid hybrid. This may be accomplished by minimizing the length of perfect complementarity to non-target organisms, avoiding G and C rich regions of homology to non-target sequences, and by positioning the probe to span as many destabilizing mismatches as possible (for example, dG:rU base pairs are less destabilizing than some others). Second, the stability of the probe:target nucleic acid hybrid should be maximized. This may be accomplished by avoiding long A and T rich sequences, by terminating the hybrids with G:C base pairs and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %G and %C result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The importance and effect of various assay conditions will be explained further herein. Third, regions of the rRNA which are known to form strong structures inhibitory to hybridization are less

preferred. Finally, probes with extensive self complementarity should be avoided."

Therefore, it would have been **prima facie** obvious to one of ordinary skill in the art at the time the invention was made to have designed probes and primers to Candida, as taught by Lott et al and Lott, to the regions of variability found between the two species such that the two species may be differentiated as taught by Hogan. The skilled artisan would have been motivated to have designed probes and primers to differentiate these Candida species for the benefit of differentiating the species from one another. The ordinary artisan would have been specifically motivated to identify and differentiate the Candida species within the ITS2 region. The knowledge in that art at the time the invention was made was extremely high for aligning sequences and finding regions of variability such that sequence comparison and differentiation between internal spacer regions to identify regions of either variability or conservation was well known and studied. There is a reasonable expectation of success for aligning known sequences, namely *Candida albicans, Candida parapsilosis, Candida tropicalis, Candida krusei*, and Candida glabrata, as taught by Lott et al with the *Candida dupliniensis* sequence of Lott using known computer alignment methods and identifying regions of variability between the species to generate probes which are species specific, as taught by Hogan. Within the alignment provided between a select group of the Candida species provided by Lott et al and Lott, the instant probes are within regions of variability. Thus, in view of the teachings in the art, a method for detecting fungal pathogenic species using the probes of SEQ ID NO: 11-12 are obvious.

13. Claims 19-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol 49, No. 1, pg M23-M28) and Botelho et al. (Yeast, Vol 10, pg 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 1-10 above or Lott et al (US Pat. 5,426,027, June 1995) and Lott (EMBL U96719, August 1997) in view of Hogan (US Pat. 5,595,874, January 1997) and applied to Claims 1-4 and 11 above, and further in view of Fujita et al. (J. of Clinical Microbiology, Vol 33, No. 4, pg 962-967, April 1995).

Neither Williams, Botelho, Lott et al, Lott, nor Hogan specifically teach detection of fungal species using a solid support.

However, Fujita et al (herein referred to as Fujita) teaches the detection of Candida species in blood using the ITS2 region of the species. Fujita specifically teaches a microtitration plate hybridization assay where digoxigenin- and biotin labeled oligonucleotide probes were detected in an EIA by capture with streptavidin-coated microtitration plates. The microtitration plates were coated with a single-stranded DNA for hybridization studies. As seen in Table 2, a matrix format against DNA from other Candida species as well as from other fungi was used. All probes were tested against all of the target DNAS so that the pattern of reactivity could be detected (pg 964). Fujita teaches that PCR products were previously detected with Southern blotting or EtBr staining of agarose gels, but these methods are less sensitive that the microtitration plate EIA. Specifically Fujita teaches that microtitration plate EIA detection of C.

albicans DNA following PCR is easier and more rapid than that by Souther blotting (pg 966, col. 1).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified methods of Williams and Botelho or Lott et al and Lott in view of Hogan with the teachings of Fujita. The ordinary artisan would have readily recognized the improvments of solid support detection as taught by Fujita for the detection of PCR amplfied DNA. The ordinary artisan would have been motivated to have detected the PCR amplified DNA using a microtitration plate EIA for the express benefits of increased sensitivity, ease and speed, as described by Fujita. Thus, the ordinary artisan would have detected the fungal pathogens using probes from variable regions of the Candida species, as taught by Williams and Botelho or Lott et al and Lott in view of Hogan, on a solid support at taught by Fujita.

14. Claims 19-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol 49, No. 1, pg M23-M28) and Botelho et al. (Yeast, Vol 10, pg 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 1-10 above or Lott et al (US Pat. 5,426,027, June 1995) and Lott (EMBL U96719, August 1997) in view of Hogan (US Pat. 5,595,874, January 1997) and applied to Claims 1-4 and 11 above, and further in view of Jordan (US Pat. 6,017,699, January 2000)

Neither Williams, Botelho, nor Hogan specifically teach detection of fungal species using a solid support.

However, Jordan teaches five species-specific primers and probes for Candida. Jordan teaches that a multiplex PCR amplfiication and agarose gel electrophoretic detection (Figure 4). Jordan teaches, in Example IV, three approaces of carrying out detection and/or confirmation of the four species of Candida (col. 16). Within these apparoaches, Jordan teaches coating a 96 well plate with biotin labelled primer for detection. Jordan also teaches that "use of the PCR master mix containing all 3 newly designated species-specific primer pairs resulted in accurate amplification of the predicted sized fragment for the DNA template added" (col. 21, lines 9-20). The multiplex approach to DNA amplification was successful. Jordan teaches significant increase in the level of sensitivity for detecting the candidal organism (Table 4)" (col. 19).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified methods of Williams and Botelho or Lott et al and Lott in view of Hogan with the teachings of Jordan. The ordinary artisan would have readily recognized the improvements of solid support detection as taught by Jordan for the detection of PCR amplified DNA simultaneously. The ordinary artisan would have been motivated to have detected the PCR amplified DNA which has a "significant increase in the level of sensitivity for detecting the candidal organism (Table 4)". Thus, the ordinary artisan would have detected the fungal pathogens using probes from variable regions of the Candida species, as taught by Williams and Botelho or Lott et al and Lott in view of Hogan, on a solid support at taught by Jordan.

15. Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Williams et al. J. Clinc. Path. Vol 49, No. 1, pg M23-M28) and Botelho et al. (Yeast, Vol 10, pg 709-717, 1994) in view of Hogan (US Pat. 5,595,874, January 1997) as applied to Claims 1-10 above or Lott et al (US Pat. 5,426,027, June 1995) and Lott (EMBL U96719, August 1997) in view of Hogan (US Pat. 5,595,874, January 1997) and applied to Claims 1-4 and 11 above, and further in view of Fujita et al. (J. of Clinical Microbiology, Vol 33, No. 4, pg 962-967, April 1995) and Tomblin et al (US Pat. 4,617,102, Ocotber 1986).

Neither Williams nor Botelho nor Lott et al nor Lott nor Hogan specifically teach isolating fungal pathogens from blood.

However, Fujita et al (herein referred to as Fujita) teaches the detection of Candida species in blood using the ITS2 region of the species. Fujita specifically teaches unlike urine or sputum, blood is a normally sterile fluid (pg 965, col. 2). Fujita teaches that Candida sp. DNA from blood, particularly from that of persistently granulocytopenic patients, raises the suspicion of deep-seated infection. Moreover, Fujita teaches that a PCR-based test for candidemia should be more sensitive than conventional blood culture methods since DNA from dead blastoconidia, as well as that from viable blastoconidia, is detected and the target sequence is amplified many fold. Fujita teaches lysing erthrocytes and leukocytes by adding 0.8 ml of TE buffer (10mM tris, 1 mM EDTA, pH 8.0) (pg 963, col. 1).

Tomblin et al (herein referred to as Tomblin) teaches a blood lysis procedure which consists of adding 0.5 ml of whole blood with 4.0 ml of DNA lysis buffer, such buffer consisting of 10mM tris-HCL, pH 7.4, 10 mM NaCl, 10 mM EDTA and mixing.

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified teachings of Williams and Botelho or Lott et al and Lott in view of Hogan to further extract the fungal pathogens from blood as taught by Fujita under conditions of Tomblin. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the conditions selected was other than routine as compared to the closest prior art. The ordinary artisan would have been motivated to have sampled blood from a patient to determine the candida status of the blood. Fujita specifically teaches that blood is blood is sterile and amenable to sensitive detection. Further the ordinary artisan would have modified the lysis procedure to obtain optimal results.

Conclusion

16. No claims allowable over the art.

17. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Enewold Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Thursday from 7:00AM to 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Jeanine Enewold Goldberg
April 4, 2001

W. Gary Jones
Supervisory Patent Examiner
Technology Center 1600

4/6/01